Relative Concentration of Cry1A in Maize Leaves and Cotton Bolls with Diverse Chlorophyll Content and Corresponding Larval Development of Fall Armyworm (Lepidoptera: Noctuidae) and Southwestern Corn Borer (Lepidoptera: Crambidae) on Maize Whorl Leaf Profiles

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ABSTRACT To manage insect resistance to transgenic crops that express insecticidal proteins from *Bacillus thuringiensis* (Bt) Berliner, the U.S. Environmental Protection Agency recommends a refuge-based insect resistance management strategy where a percentage of non-Bt (refuge) crop is grown in proximity to a Bt-expressing crop. An important requirement for this strategy is that the toxin exists at a high effective dose for control of the target pest(s), so that heterozygous individuals in the population do not reach adulthood. Factors that cause reduced levels of toxin in the plant are a threat to this strategy. We quantified Cry1Ab from different areas of the maize, *Zea mays* L., leaf. In general, the distal tip of the V7 maize leaf had a higher concentration of Cry1Ab compared with the middle section of the V7 leaf, and the middle section of the developing V9 leaf had the lowest concentration of Cry1Ab. When these sections of maize tissue were fed to fall armyworm, *Spodoptera frugiperda* (J.E. Smith), and southwestern corn borer, *Diatraea grandiosella* Dyar, there was not a reduction in development or an increase in mortality with tissue that had higher concentrations of toxin. Another study tested the relative concentration of Cry1Ab between the white-yellow, yellow-green, and green portions of the developing ninth leaf within the maize whorl. There were differences in Cry1Ab concentration among these leaf areas. The green tissue had the highest concentration of toxin followed by the yellow-green and white-yellow tissues. Correlations between concentration of Cry1Ab and 5-d fall armyworm larval weights among the three leaf color profiles were all significant and negative, i.e., decreased concentration of Cry1Ab in the leaf tissue resulted in increased 5-d larval weights. There was 100% mortality to the southwestern corn borer larvae fed Cry1Ab maize leaf tissue. Differences in the amount of Cry1Ab in the developing V9 leaf profiles did not alter the absolute susceptibility of the southwestern corn borer to the toxin. In cotton, *Gossypium hirsutum* L., the amount of Cry1Ac was significantly lower in boll tips where flowers had remained attached compared with normal boll tips. Boll tips where the flowers remained attached are often the site where corn earworms, *Helicoverpa zea* (Boddie), penetrate Bt cotton bolls. This study demonstrated that, in two diverse plant species, tissue that has low chlorophyll content does not fully express Cry1A. Photosynthesis regulating factors related to mRNA transcription and translation should be studied for their effect on Cry1A production and insect control.

KEY WORDS *Spodoptera frugiperda*, *Diatraea grandiosella*, *Bacillus thuringiensis*, *Zea mays*, *Gossypium hirsutum*

FROM 1996 TO 2003, THE MOST commonly grown insect-resistant transgenic maize, *Zea mays* L., hybrids and cotton, *Gossypium hirsutum* L., varieties have expressed crystalline proteins Cry1Ab (event MON810) and Cry1Ac (event MON531), respectively, from the bacterium *Bacillus thuringiensis* Berliner (Bt) (Koziel et al. 1993, Shelton et al. 2002). The transformation events MON810 and MON531 results in toxin expression in all plant tissues, albeit at different levels (Armstrong et al. 1995, Greenplate 1999, Adamczyk et al. 2001). Within-plant variability of toxin produced in maize and cotton may alter the level of control for some of the targeted lepidopteran pests of these crops. Maize and cotton plants that use these two transformation events were examined in this study for their production of Cry1A toxin.

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The sustained expression of Bt toxins by transgenic plants during entire growing seasons will likely increase the frequency/intensity of selection for Bt resistance in lepidopteran insect populations. Resistance to Bt endotoxins has already been observed in laboratory colonies and field populations of some insects (Tabashnik 1994, Chaufaux et al. 1997, Perez and Shelton 1997, Gould 1998, Huang et al. 1999, Burd et al. 2000, Tabashnik et al. 2000). The U.S. Environmental Protection Agency recommends a refuge-based insect resistance management strategy (EPA 1998) where a percentage of non-Bt (refuge) crop is grown in proximity to a Bt-expressing crop. This refuge is designed to produce enough susceptible insects so that resistant × resistant matings are unlikely to occur. An important requirement for this strategy is the expression of toxin at a high effective dose, so that heterozygous individuals in the population do not reach adulthood.

A threat to this refuge-based strategy are factors that cause lower concentrations of Bt toxin in the plant. Heterozygous individuals are more likely to develop into adults on plants that have a low concentration of Bt toxin. During the initial development of resistance in a population, when resistance alleles are rare, heterozygous individuals carry the majority of the resistance to Bt endotoxin genes. Models of resistance evolution indicate survival of insects with one copy of a resistance gene could greatly accelerate selection for resistant populations (EPA 1998). Adamczyk et al. (2001) found differential tissue and temporal expression of δ-endotoxin in cotton and a high negative correlation between survival and development of corn earworm, *Helicoverpa zea* (Boddie), larvae. Bruns and Abel (2003) discovered lower rates of nitrogen fertilization in corn reduced the levels of Cry1Ab in maize whorl leaf tissue.

For our study, the first objective was to quantify the relative concentration of Cry1Ab present in sections of maize leaf tissue that had visibly different levels of chlorophyll. Our second objective was to correlate lepidopteran larval development to the relative amount of Cry1Ab present in the different sections of maize leaf tissue. Our final objective was to quantify the relative differences in the amount of Cry1Ac in cotton boll tissue with diverse chlorophyll content. In particular, because the boll tip area under an attached flower corolla is often a site for lepidopteran penetration into the cotton boll, we hypothesized that the Cry1Ac levels were lower under these conditions compared with normal bolls.

Materials and Methods

Maize. Transgenic maize hybrids and their closely related isolines (Table 1) were planted in Stoneville, MS, during the summers of 1999 and 2000 on 23 April and 21 March, respectively. Forty-two seeds from each of the hybrids were planted in an individual row for each experimental unit and thinned to 30 plants. Standard maize production procedures for the area were used. Maize hybrids were planted in rows that were 6.1 m in length and 1.0 m apart in a randomized complete block arrangement with four replications. In each year, two studies were planted separately, one for testing resistance to fall armyworm and the other for testing resistance to southwestern corn borer. Maize plants were tested at the V7 stage (Ritchie et al. 1992) because infestations of fall armyworm, *Spodoptera frugiperda* (J.E. Smith), on maize whorls (Ditman 1950, Morrill and Greene 1973) typically occur at or near this stage in the Mississippi Delta. Infestations of southwestern corn borer, *Diatraea grandiosella* Dyar, on maize whorls (Davis et al. 1972) typically occur later than fall armyworm infestations but can occur at the V7 stage or earlier in the Mississippi Delta, depending upon seasonal variation effects on pest phenology and maize planting dates. All fall armyworms and southwestern corn borers used for this research were obtained from a culture maintained by the USDA–ARS, Corn Host Plant Resistance Research Unit (Mississippi State, MS) by using the technique described by Davis (1989). Wild adults are collected from the field and interbred with the existing culture annually.

Table 1. Commercially available maize hybrids and cotton varieties tested at Stoneville, MS, and their transformation events

	Cry toxin	Transformation event	Closely related non-Bt isoline
Corn Hybrid			
Agrigold A6609Bt	Cry1Ab	MON810	Agrigold XA3713
Asgrow RX799Bt	Cry1Ab	MON810	
Dekalb DK679BtY	Cry1Ab	MON810	Dekalb DK679
Pioneer brand 31B13	Cry1Ab	MON810	Pioneer brand 3223
Pioneer brand 33V08	Cry1Ab	MON810	Pioneer brand 3394
Cotton variety			
NuCOTN 33B	Cry1Ac	MON531	DP5415
NuPEARL	Cry1Ac	MON531	DPPEARL
ST 4691B	Cry1Ac	MON531	ST474
DP 458BR	Cry1Ac	MON531	DP5415
DP50B	Cry1Ac	MON531	DP50

1999 Test. At the V7 stage (Ritchie et al. 1992) of maize growth, the seventh leaf, from three plants within each of four rows (blocks) per hybrid entry, were excised from the plant at the leaf axil ($n = 48$ leaves, three leaves per four blocks per four hybrids). The developing ninth leaf was harvested from the same test plants by grasping the leaf within the whorl and pulling it up, thus removing it from the plant. The harvested leaves were placed in a labeled paper bag and taken to the laboratory. An approximate 6.0 cm long by leaf-width section was excised from the approximate center and the distal tip of the seventh true leaf and from the approximate center of the developing ninth leaf. From these sections, an approximately 20-mg portion of leaf was excised from the leaf margin of each section, weighed, placed into a 1.5-ml microcentrifuge tube that was capped, and frozen at −80°C for analysis of Cry1Ab. The remaining 6.0-cm leaf

sections were divided at the midrib into two halves. Each half was then positioned with the adaxial side of the leaf up and placed into a petri dish (10 by 100 mm) previously prepared with 1% agar that was poured as a thin layer covering the bottom of each dish as described by Abel and Wilson (2000). A single fall armyworm or southwestern corn borer neonatal larva was then placed in each dish. The petri dish was covered with a lid and sealed with Parafilm (American Can Company, Greenwich, CT) to prevent desiccation of the leaf tissue and escape of the larvae. The dishes were then placed into a growth chamber (model I-36 LLX, Percival Scientific, Boone, IA) kept at a constant 26.7 ± 2°C and 60 ± 5% RH in a photoperiod of 16:8 (L:D) h. Larval mortality and 5-d weights were recorded. The bioassay experiment was arranged as a randomized complete block design with four replicates based on blocks in the field. For the larval bioassays, six subsamples (petri dishes) were assigned to each experimental unit. The leaf sections were randomly assigned to the petri dishes. Larval mortality, larval weight, and Cry1Ab concentration in ppm were analyzed using REML-analysis of variance (ANOVA), and means were separated with the LSMEANS option of PROC MIXED (SAS Institute 1995, Littel et al. 1996).

Within 1 mo, the microcentrifuge tubes containing the maize tissue were removed from −80°C. The excised leaf samples were homogenized by hand within the tubes by using Cry1Ab/Ac extraction buffer (EnviroLogix, Inc., Portland, ME) and a fitted pestle. To quantify the relative amount of Cry1Ab present for each tissue sample, a commercial enzyme-linked immunosorbent assay (ELISA) kit was used (EnviroLogix, Inc.). Quantification of Cry1Ab was determined photometrically (Benchmark, Bio-Rad, Hercules, CA). A standard curve was established using known quantities of Cry1Ab. Dilution factors, positive and negative controls, and calculations were conducted as dictated in the kit protocol and Abel and Pollan (2004) and Adamczyk et al. (2001). Relative Cry1Ab levels (ppm) were analyzed using REML-ANOVA, and means were separated with the LSMEANS option of PROC MIXED (SAS Institute 1995, Littel et al. 1996). Non-Bt leaf samples were included throughout the study and used as negative controls.

2000 Test. For the larval bioassays and Cry1Ab quantification, the developing ninth leaf from V7 stage plants (Ritchie et al. 1992) was harvested by grasping the seventh leaf at the leaf axil and removing the seventh leaf and all developing leaves within the whorl from the plant. This harvested portion of the plant was immediately placed into a cooler that was prepared with water ice, and within 1 h of harvest, the cooler was taken to the laboratory and the harvested portion was unwrapped to remove the developing ninth leaf. This leaf was typically green along the distal half of the leaf (exposed to full sunlight in the field), white-yellow along the basal quarter of the leaf (exposed to little or no sunlight in the field), and yellow-green in between the green and white-yellow sections. The developing ninth leaf was harvested in this way from 14 plants for each of the nine maize hybrids.

A 6.0 cm long by leaf-width section was excised from the approximate center of each color portion. From these sections, a cork borer was used to excise a 2-cm-diameter leaf disk directly next to, but not including, the leaf midrib. The leaf disk was then frozen at −80°C and stored for toxin analysis. The remaining 6.0-cm leaf section was divided at the midrib into two halves. The undamaged half was then placed adaxial side up into a petri dish previously prepared with 1% agar and infested with a single larvae as described for the 1999 test. The dishes were then placed into a growth chamber (model I-36 LLX, Percival Scientific) kept at a constant 26.7 ± 2°C and 60 ± 5% RH in a photoperiod of 16:8 (L:D) h. Larval mortality and larval weights were recorded after 5 d. The bioassay experiment was arranged as a randomized complete block design with 14 replicates for each color portion of each of the nine hybrid entries. A replication was a group of three petri dishes (containing three different color-profile leaves) that were stacked on top of each other and placed inside the growth chamber. Leaf sections from each of the color profiles were randomly assigned to the three petri dishes (replicate). Larval mortality, larval weights, and Cry1Ab concentration in ppm were analyzed using REML-ANOVA, and means were separated with the LSMEANS option of PROC MIXED (SAS Institute 1995, Littel et al. 1996).

Approximately 20 mg of tissue was removed and weighed from each frozen 2-cm-diameter leaf disk to quantify the amount of Cry1Ab present. A non-Bt variety (Pioneer brand 3394) was used as a negative control. Cry1Ab quantification was conducted as described for the 1999 test. Relative Cry1Ab concentration in ppm were analyzed using REML-ANOVA, and means were separated with the LSMEANS option of PROC MIXED (SAS Institute 1995, Littel et al. 1996).

Cotton. Transgenic Bt cotton varieties (five) that express Cry1Ac were planted in experimental plots arranged in a randomized complete block design (Table 1). A 18.3 by 27.4-m cage was erected, and the entire test was covered with netting. This allowed a considerable reduction of sunlight while maximizing humidity, causing a large percentage of flowers to remain attached to boll tips. For each plot, normal bolls and bolls containing partially detached blooms (five each per variety) were collected and transported to the laboratory. The tips were dissected away from the remaining boll material, pooled for each variety, and the amount of Cry1Ac quantified as described above for maize. Because only Cry1Ab calibrators are supplied with the kit, a simple conversion was used to express values as "Cry1Ac" as dictated by the kit protocol. Means were analyzed using REML-ANOVA, and were separated with the LSMEANS option of PROC MIXED (SAS Institute 1995, Littel et al. 1996).

Results and Discussion

Maize. *Fall Armyworm Tests.* In 1999, there were differences among the three leaf locations (middle

Table 2. Mean ± SEM concentration of Cry1Ab in ppm for leaf tissue from the middle and distal tip of the V7 maize leaf and the middle of the developing V9 maize for a fall armyworm (FAW) and southwestern corn borer (SWCB) study, Stoneville, MS, 1999

Leaf tissue	P31B13		P33V08		RX799Bt		A6609Bt	
	FAW study	SWCB study	FAW study	SWCB study	FAW study	SWCB study	FAW study	SWCB study
Middle V9	0.73 ± 0.13a	0.66 ± 0.11a	0.66 ± 0.01a	0.76 ± 0.04a	0.90 ± 0.10a	0.77 ± 0.17a	0.92 ± 0.13a	0.76 ± 0.11a
Middle V7	1.24 ± 0.21b	0.77 ± 0.04a	0.68 ± 0.06a	1.13 ± 0.20b	1.22 ± 0.07a	1.28 ± 0.10b	0.92 ± 0.16a	1.33 ± 0.18b
Distal tip V7	2.13 ± 0.12c	2.20 ± 0.13b	1.93 ± 0.16b	2.17 ± 0.10c	2.16 ± 0.19b	2.39 ± 0.11c	2.22 ± 0.03b	2.30 ± 0.22c

Means followed by the same letter are not significantly different according to the least significant difference test ($P < 0.05$).

and distal tip of the V7 leaf and the middle of the developing V9 leaf) for the relative level of Cry1Ab across Bt hybrids ($F = 115.88$; df = 2, 33; $P < 0.0001$). There were no differences between Bt hybrids × leaf location ($F = 1.08$; df = 6, 33; $P = 0.3921$). For Bt hybrid Pioneer brand 31B13 (P31B13), there was more Cry1Ab expressed in the distal tip of the seventh leaf compared with the middle portion of the seventh leaf and more Cry1Ab expressed in the middle portion of the seventh leaf compared with the developing ninth leaf (Table 2). For the remaining Bt hybrids tested, there was more Cry1Ab expressed in the distal tip of the seventh leaf compared with the middle portions of the seventh and developing ninth leaves (Table 2). ANOVA for the laboratory bioassay showed no differences in 5-d fall armyworm larval weights between the leaf locations for the Bt hybrids ($F = 2.05$; df = 2, 32; $P = 0.1459$). There were differences in percent larval mortality between the leaf locations for the Bt hybrids ($F = 33.15$; df = 2, 33; $P < 0.0001$); however, the results were difficult to explain, with the lowest Cry1Ab expressing leaf tissue (middle V9) having the highest larval mortality (71.5%) compared with the middle V7 and tip V7 tissue (39.3 and 17.6%, respectively). For the non-Bt hybrids, there was no difference in larval mortality for the three leaf sections ($F = 1.81$; df = 2, 21; $P = 0.1875$).

In 2000, the ANOVA for ppm Cry1Ab contained in white-yellow, yellow-green, and green portions of the developing ninth leaf showed differences among these leaf areas ($F = 43.20$; df = 2, 204; $P < 0.001$). The green tissue had the highest amount of Cry1Ab followed by the yellow-green tissue and the white-yellow tissue having the lowest amount (Table 3). The yellow-green and white-yellow portions of the developing maize leaf are where the fall armyworm typically feeds (Morrill and Greene 1973). Correlations between ppm of Cry1Ab and 5-d larval weights among the three leaf color profiles that had live larvae after 5 d were all significant and negative (Fig. 1). Decreasing levels of Cry1Ab in the leaf tissue resulted in increased 5-d larval weights. Adamczyk et al. (2001) found a correlation between reduced expression of Cry1Ac in cotton and increased survival of corn earworm. Whorl leaf profile and other maize morphological effects on the production of Cry1Ab proteins should be investigated further to determine whether lower toxin levels correlate with a reduction in fall armyworm control. Amplification of Cry1Ab being produced in the plant or research integrating other control methods with the use of Cry1Ab may be needed to protect the crop from economically important damage by the fall armyworm.

Table 3. Mean ± SEM ppm Cry1A in white-yellow, yellow-green, and green portions of developing V9 maize leaf for a fall armyworm (FAW) and southwestern corn borer (SWCB) study, Stoneville, MS, 2000

Leaf tissue	Concentration of Cry1A (ppm)	
	FAW study	SWCB study
White-yellow	0.18 ± 0.01a	0.22 ± 0.07a
Yellow-green	0.33 ± 0.03b	0.33 ± 0.06a
Green	0.60 ± 0.05c	0.59 ± 0.07b

The primary insect resistance management strategy, advocated by the U.S. Environmental Protection Agency for Bt crops, recommends that plants express the δ-endotoxins at high levels so that insect resistance to the toxins is functionally recessive (EPA 1998). Plant tissue with reduced concentrations of toxin could allow heterozygous individuals to survive. These individuals carry the majority of the resistance alleles to Bt endotoxins. Models of insecticide resistance evolution suggest that survival of these heterozygous individuals, which carry one copy of a resistance allele, could greatly accelerate selection for resistant populations (EPA 1998). Research should be conducted to determine whether the reduced levels of Cry1Ab that were discovered in maize leaf profiles is a threat to the high-dose strategy recommended by the Environmental Protection Agency.

Southwestern Corn Borer Tests. The ANOVA for ppm Cry1Ab showed differences among the three leaf locations (middle and distal tip of the V7 leaf and the middle of the developing V9 leaf) ($F = 133.71$; df = 2, 33; $P < 0.001$) and no difference among Bt hybrids ($F = 2.45$; df = 3, 33; $P = 0.0811$) and Bt hybrids × maize leaf locations ($F = 0.80$; df = 6, 33; $P = 0.5750$) in 1999. For Bt hybrids Pioneer brand 33V08 (P33V08), RX799Bt, and A6609Bt, there was more Cry1Ab expressed in the distal tip of the seventh leaf compared with the middle portion of the seventh leaf and more Cry1Ab expressed in the middle portion of the seventh leaf compared with the middle portion of the developing ninth leaf (Table 2). For P31B13, there was more Cry1Ab expressed in the distal tip of the seventh leaf compared with the middle portions of the seventh and developing ninth leaves (Table 2). There was 100% larval mortality across all Bt hybrids × maize leaf locations ($n = 137$) compared with 7.0% mortality on conventional hybrid leaf tissue ($n = 71$). Previous research also has demonstrated a high degree of

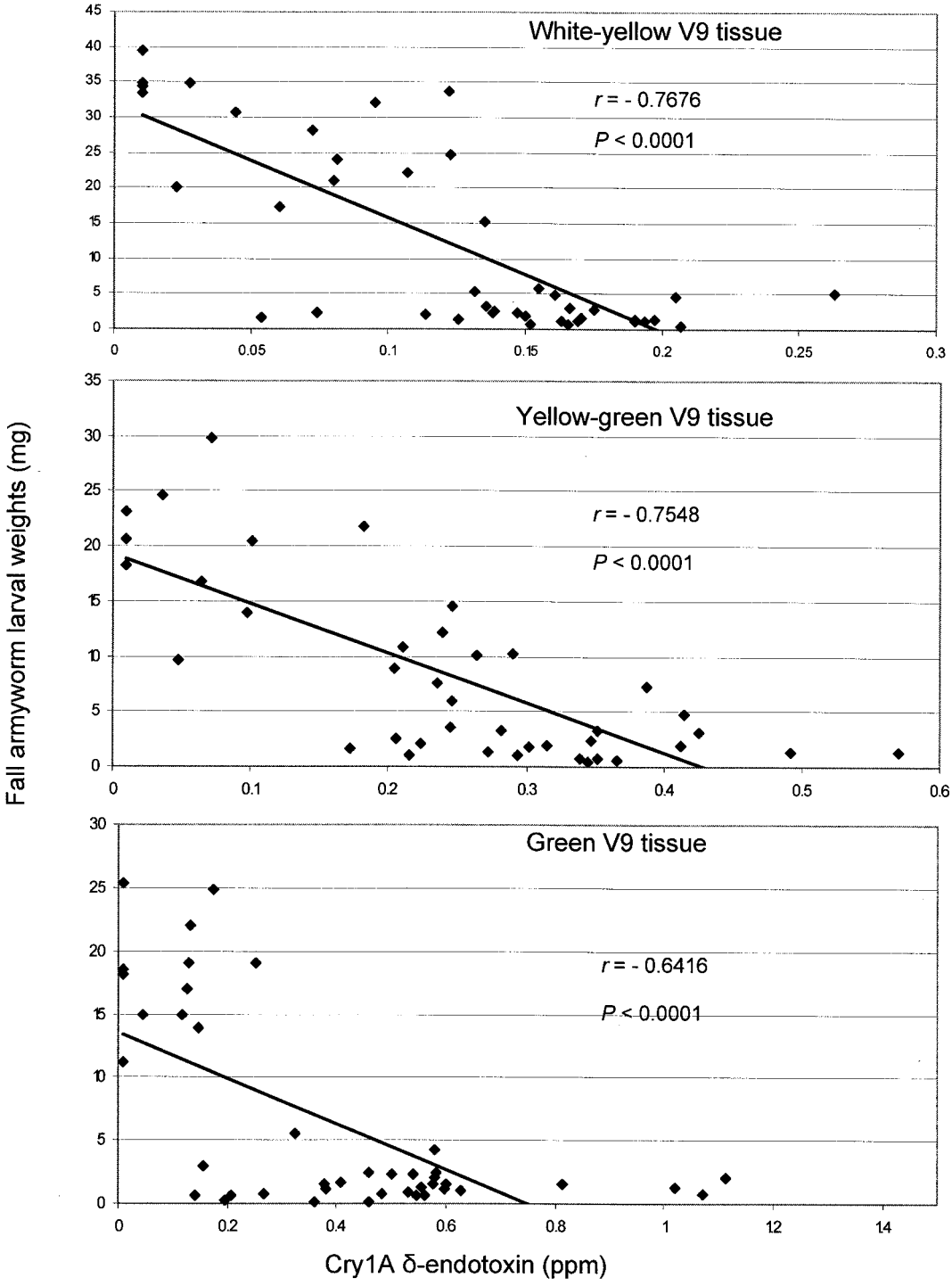

Fig. 1. Correlation and trend lines of Cry1Ab δ-endotoxin (ppm) in white-yellow, yellow-green, and green profiles of the developing V9 maize whorl leaf from four Bt maize hybrids and 5-d larval fall armyworm weights (milligrams). Data points are from leaf samples that had one live larvae after 5 d.

Cry1Ab susceptibility in southwestern corn borers (Williams et al. 1997, Abel and Pollan 2004).

The ANOVA for ppm Cry1Ab showed differences among maize whorl leaf profiles ($F = 11.42$; df = 2, 69; $P < 0.0001$) in 2000. The green leaf profile contained more Cry1Ab than the yellow-green and white-yellow

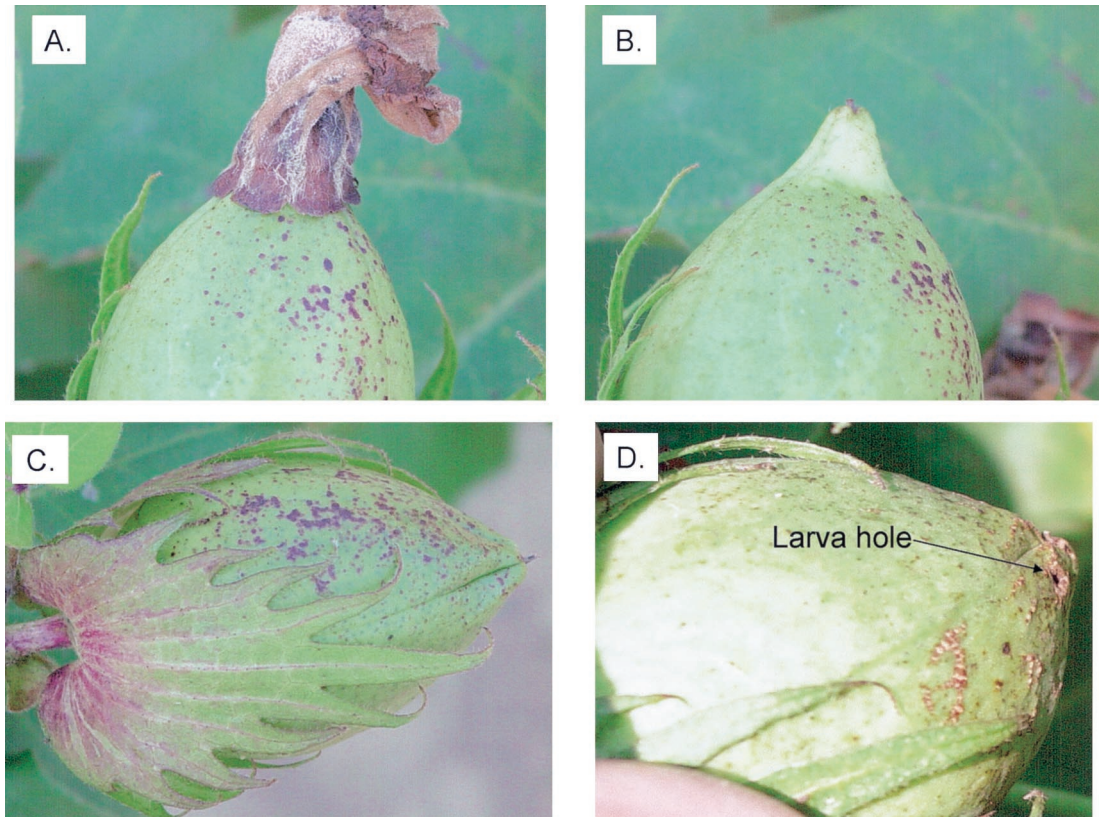

Fig. 2. Types of cotton bolls used for Cry1Ac quantification. (A) Cotton boll with flower corolla attached to the boll tip. (B) Same boll with flower corolla removed from boll tip. (C) Normal cotton boll. (D) Heliothine-damaged boll tip.

profiles (Table 3). The white-yellow portion of the developing maize leaf is where early instars of southwestern corn borer typically feed for ≈10 d before boring into the stalk of the plant (Hensley and Arbuthnot 1957, Davis et al. 1972). There were no surviving larvae after 5 d for the Cry1Ab-expressing hybrids. Differential amounts of Cry1Ab in the developing V9 leaf did not alter the absolute susceptibility of the southwestern corn borer to the toxin.

Cotton. The amount of Cry1Ac was significantly lower ($F = 33.25$, df = 1, 4, $P = 0.004$) in boll tips where flowers had remained attached (Fig. 2) compared with normal bolls (attached flowers, 1.47 ppm ± 0.070; normal bolls, 1.68 ppm ± 0.101; Fig. 3). Although we did not test chlorophyll content for boll material under partially detached corollas, it was visibly obvious that chlorophyll content was lower compared with normal boll tips (i.e., white). Corn earworms are often

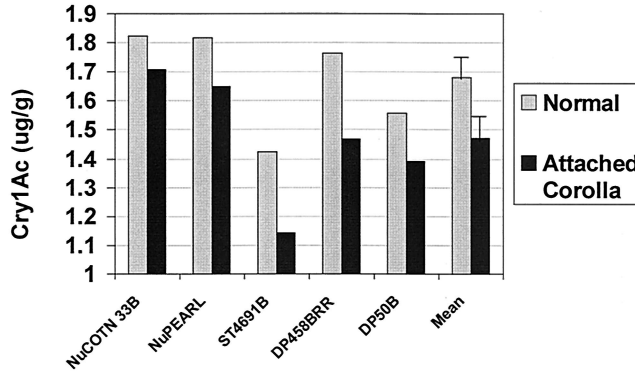

Fig. 3. Amount of Cry1Ac from normal boll tips and boll tips where the flower corolla was attached for five varieties of Bt cotton.

found feeding in cotton flowers, and once the flowers begin to senesce, begin feeding on bolls (Gore et al. 2003). In addition, boll tips are often the site where corn earworms penetrate Bt cotton bolls (J.J.A., unpublished). Therefore, it is possible that Bt cotton bolls, where the corresponding flower remains attached, have more damage than normal bolls. This damage is due in part to the reduced amount of Cry1Ac, although more research needs to be conducted.

***Cry1A* Transcription and Translation.** This study has demonstrated that two genetically transformed plant species from two classes (class Liliopsida, i.e., monocotyledonous plants, and class Magnoliopsida, i.e., dicotyledonous plants) did not fully express Cry1A in tissue that had low chlorophyll content. A number of factors influence a plant tissue's ability to produce chlorophyll to prepare for photosynthesis. Some of these factors for most plant species include exposure to sunlight and mineral deficiencies. Bruns and Abel (2003) correlated nitrogen deficiencies with reduced levels of Cry1A in plant tissue. Production of Cry1A toxin is likely related to the plants ability to produce precursors (i.e., amino acids) via photosynthesis that are used to produce the Cry1A insecticidal proteins. Therefore, other factors that limit photosynthesis (e.g., reduced quality and duration of sunlight; low carbon dioxide concentration; low chlorophyll production, which is usually related to mineral deficiencies; low available water; high or low temperatures; unbalanced oxygen/carbon dioxide ratios; pollution; and specific photosynthesis inhibitors such as dichlorophenol dimethyl urea, which is used in some herbicides; Kerrison 1998) also may limit Cry1A production in genetically transformed plant species and should be investigated in the future.

The interaction between the factors that regulate photosynthesis and the production of Cry1A may become an important area of study. For example, Lawler et al. (1997) found that elevated carbon dioxide levels increased photosynthesis, which lead to higher carbon/nitrogen ratios. This resulted in lower nitrogen levels per unit of plant tissue (Curtis and Wang 1998). Coviella et al. (2000) found that this lower nitrogen content from elevated atmospheric carbon dioxide actually decreased Cry1A production in cotton plants. This demonstrates the importance of studying the interactions between photosynthesis regulating factors for their effect on Cry1A production and insect control.

Other factors that affect transcription of mRNA that encodes for Cry1A production may enhance or limit the plant's ability to produce the toxin at optimal levels. In eukaryotic cells, an extensive and complex system of factors regulates transcription of mRNA. This system is more complex in C_4 plants (e.g., maize), which requires the cooperation of two different cell types to complete C_4-based photosynthesis (Hatch 1977). Some of these factors that produce translatable mRNA, e.g., phosphoenolpyruvate carboxylase kinase in maize leaves, are increased in direct response to light (Hartwell et al. 1996). Because we found less Cry1A toxin being expressed in plant tissue that was not fully exposed to sunlight, mRNA transcription factors that are regulated by light should be investigated to determine their effect on Cry1A production in maize and cotton tissue.

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